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Mutagenicity of Selected Organic Solvents

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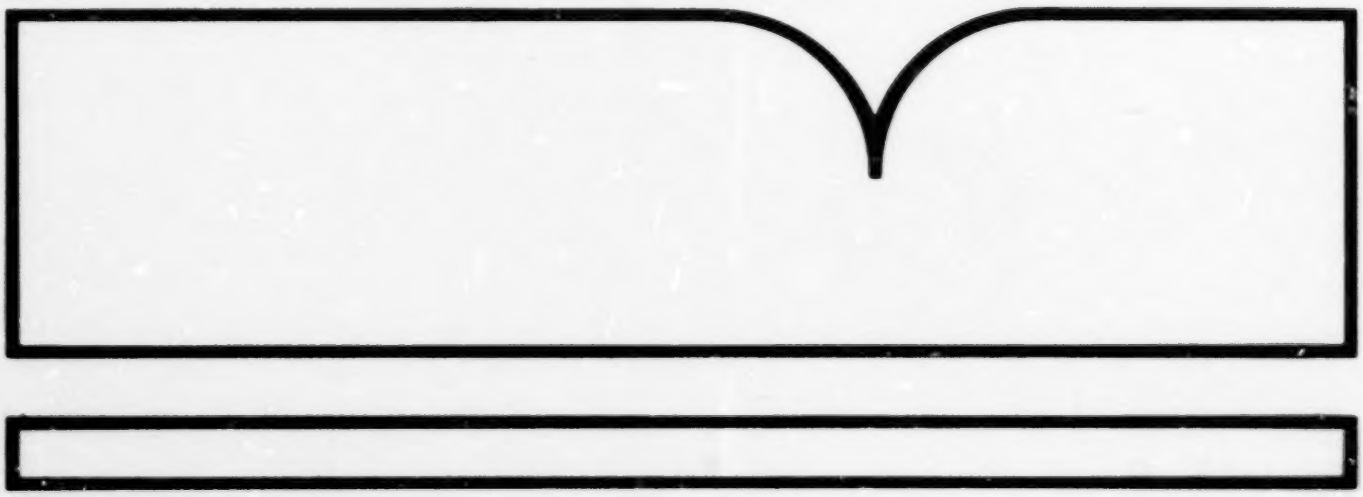

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Mutagenicity of selected organic solvents

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VAINIO H, WATERS MD, NORPPA H. Mutagenicity of selected organic solvents. *Scand J Work Environ Health* 11 (1985): suppl 1, 75—82. For certain organic solvents, such as benzene, vinyl chloride, styrene, technical grade trichloroethylene, and acrylonitrile, the available studies provide convincing evidence to demonstrate activity in short-term genetic assays. For a few solvents, such as phenol, vinyl toluene, ethanol, and tetrachloroethylene, the evidence is limited to a certain test system and/or test organism. For most of the solvents reviewed, studies are either lacking or they are so inadequate that no final evaluation on the mutagenic activity of the solvents can be made.

Key terms: chromosome aberrations, genetic activity, micronuclei, mutagenicity, organic solvents.

Among industrial chemicals, organic solvents are of major concern because, generally, they are produced in large volume, have broad use patterns, and are disseminated in both the workplace and the general environment. Hence, there is wide potential population exposure. Over the past three decades, there has been a vast increase in the production and use of organic solvents. Their uses are multifarious, and they are found in reaction media, extractants, dry-cleaning fluids, paints, coatings, adhesives, and sealants. They belong principally to the following structural classes: aliphatic, aromatic and chlorinated hydrocarbons, alcohols, ketones, ethers, nitroso compounds, aldehydes, and phenols.

In recent years there has been increasing concern about the potential mutagenicity, carcinogenicity, and teratogenicity of organic solvents. At present, more than 100 test systems exist for the evaluation of chemicals that produce genetic or genetic-related effects; however, analysis of the complete data base on the genetic toxicology for solvents is difficult due to the large number of test systems and the large number of solvents in use.

In this review, we have used genetic activity spectra (14) to summarize the qualitative and quantitative data available in the literature on selected organic solvents. In this approach, information on the lowest effective dose or the highest ineffective dose is recorded for each solvent and assay system. The assay systems are classified by type of genetic activity measured and subdivided by the phylogenetic level of the test organism. The quantitative results obtained in the test systems are represented by computer-generated bar graphs. [For details of the analysis of the genetic activity spectra, see Garrett et al (14).]

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Representation of genetic activity spectra for solvents

A data set consisting of a discrete number of assays and the doses required to produce responses in the assays is represented in a bar graph. This representation has been accomplished with the use of x-axis unit values corresponding to 100 different test systems and y-axis values corresponding to qualitative responses and quantitative estimates of the doses employed to obtain the responses for each test system. The numbers and corresponding codes assigned to the different assays on the x axis are given in table 1 (14).

The term "dose" as used in this presentation does not take into consideration treatment or exposure time and may therefore be considered synonymous with concentration. Doses and concentrations are reported in the literature in various units (eg, as parts per million, percentage, mass per volume, volume per volume). For comparative assessment, all units have been converted to mass per volume. Dose units for in vitro test systems are expressed as micrograms per milliliter; for microbial plate-incorporation tests, a volume of 2 ml is assumed for the top agar, and a 1-ml volume is taken for differential toxicity assays. Doses used in in vivo bioassays are expressed as milligrams per kilogram of body weight of the treated animal.

Benzene and its derivatives

Benzene is leukemogenic to humans (18) and produces tumors in various organs in rodents (16, 27). It also induces chromosome aberrations, micronuclei, and sister chromatid exchange (SCE) in mammalian cells in vitro and in vivo, but there is only one positive report

Table 1. Genetic bioassays used to score for genotoxicity.[a]

Number	Code	Genetic end point
POINT/GENE MUTATION ASSAYS		
Prokaryotic assays		
		Salmonella typhimurium, reverse mutation
1	SA5	TA1535
2	SA7	TA1537
3	SA8	TA1538
4	SA9	TA98
5	SA0	TA100
		Escherichia coli, reverse mutation
6	WP2	WP2
7	WPU	WP2 uvrA
8	(ECO)	Miscellaneous strains
Lower eucaryotic assays		
		Saccharomyces cerevisiae
9	YEF	Forward mutation
10	YER	Reverse mutation
		Schizosaccharomyces pombe
11	YEY	Forward mutation
12	YEZ	Reverse mutation
		Aspergillus nidulans
13	ASF	Forward mutation
14	ASR	Reverse mutation
Higher eucaryotic in vitro assays		
		Chinese hamster cells, forward mutation
15	(CHL)	Lung HGPRT locus
16	CHO	Ovary HGPRT or ATPase locus
17	V7H	Lung (V-79) HGPRT locus
18	V7O	Lung (V-79) ATPase locus
		Mouse lymphoma (L5178Y) cells
19	L5T	Forward mutation, TK locus
Higher eucaryotic in vivo assays		
20	ARM	*Arabidopsis* mutation
21	TRM	*Tradescantia* mutation
22	SRL	*Drosophila melanogaster* sex-linked recessive lethal test
23	MST	Mouse spot test
24	SLP	Mouse specific locus test, postspermatogonial stages
25	SLT	Mouse specific locus test, all stages
Host-mediated and body fluid assays		
26	HMA	Host-mediated assay
27	BFU	Body fluids, urine
OTHER GENOTOXIC EFFECTS		
Prokaryotic assays		
28	SAR	*Salmonella typhimurium*, differential toxicity assay
		Escherichia coli Pol A (W3110-P3478)
29	REP	Spot test
30	RET	Liquid suspension test
31	REW	*Bacillus subtilis* rec (H17-M45), spot test
32	REC	DNA-repair-deficient bacteria
33	(WPR)	*Escherichia coli* WP100 uvrA rec or other rec strains
Lower eucaryotic assays		
		Saccharomyces cerevisiae
34	YEC	Gene conversion
35	YEH	Homozygosis (through recombination or gene conversion)

(continued)

Table 1. Continued.

Number	Code	Genetic end point
Higher eucaryotic assays		
		Unscheduled DNA synthesis in mammals
36	(UDB)	Human bone marrow
37	UDH	Human diploid fibroblasts
38	(UDL)	HeLa cells
39	UDS	All cell types, in vitro
40	(UDX)	*Xeroderma pigmentosum* cells
41	(UDR)	Rat, in vivo
42	(UDM)	Mouse, in vivo
		Inhibition of DNA synthesis
43	(IDL)	HeLa cells
44	(IDH)	Human cells
45	(IDR)	Rodent cells
46	(DBH)	DNA strand break, human
CHROMOSOMAL EFFECTS ASSAYS		
Sister Chromatid Exchange		
47	SCC	Chinese hamster ovary (CHO) cells, transformed
48	SCF	Human fibroblasts, normal
49	SCH	HeLa cells, transformed
50	SCM	Human lymphoblastoid cells, transformed
51	SCP	Chinese hamster fibroblasts, transformed
52	SCV	Chinese hamster lung fibroblasts (V-79 cells), transformed
53	SCE	In vitro and in vivo
54	SCL	Human lymphocytes in vitro
55	SC2	In vitro, all animals except human
56	SC3	In vivo, all animals except human
57	SC4	In vivo, human cells
Aneuploidy		
58	NEN	*Neurospora crassa*
		Drosophila melanogaster
59	DAC	Whole sex chromosome loss
60	DAP	Partial sex chromosome loss
Chromosome aberrations in vitro		
		Mammalian cytogenetics
61	CYU	Chinese hamster
62	CYV	Syrian golden hamster
63	CYY	Mouse
64	CYZ	Human
65	CYC	All cell types
66	CYH	Lymphocytes, human
67	(CYX)	*Xeroderma pigmentosum* cells
Chromosome aberrations in vivo		
		Mammalian cytogenetics
68	CYB	Bone marrow studies, all animals
69	CYL	Lymphocyte or leukocyte studies, all animals
70	CYG	Spermatogonial stem cells treated, spermatocytes observed
71	CYO	Oocyte or early embryo studies
72	CYS	Spermatogonia treated, spermatogonia observed
73	CYT	Differentiating spermatogonia or spermatocytes treated, differentiating spermatocytes observed
74	HOC	*Hordeum* cytogenetics
75	TRC	*Tradescantia* cytogenetics
Micronuclei		
76	(MNC)	In vitro
		In vivo
77	(MNH)	Hamster
78	(MNM)	Mouse
79	(MNR)	Rat

(continued)

Table 1. Continued.

Number	Code	Genetic end point
Chromosome damage in vivo		
		Dominant lethal test
80	(DLD)	*Drosophila melanogaster*
81	DLM	Mouse
82	DLR	Rat
		Heritable (reciprocal) translocation
83	DHT	*Drosophila melanogaster*
84	(MHT)	Mouse
CELLULAR TRANSFORMATION ASSAYS		
		Syrian hamster embryo
85	CTC	Clonal assay
86	CTF	Focus assay
87	CTS	Transformation strains
88	CTB	BALB/c3T3 cells
89	CTH	C3H10T1/2 cells
90	CTL	Established cell lines
91	CTK	AKR/ME cells
92	CTR	RLV/Fischer rat embryo cells
93	CTV	Virus enhancement
94	CT7	SA-7/SHE cells
SPERM MORPHOLOGY ASSAYS		
95	SPA	Rat
96	SPH	Human
97	SPI	Mouse
98	SPR	Rabbit
99	SPS	Sheep
100	SPF	Mouse F_1 assay

[a] DNA = deoxyribonucleic acid, ATPase = adenosine triphosphatase, HGPRT = hypoxanthine-guanine phosphoribosyltransferase.

— in a *Tradescantia* stamen hair assay — on the induction of gene mutation by benzene (13, 41). The genetic activity spectrum of benzene is given as figure 1.

Mixed function oxidases convert benzene into benzene oxide, which has been suspected of being the important reactive metabolite (42). Benzene oxide is converted into phenol or catechol via benzene dihydrodiol, and phenol may be further metabolized to hydroquinone and catechol (39). The hypothesis that benzene oxide is the reactive species of benzene has recently been questioned, however, in reports that suggest that other metabolites of phenol, catechol and hydroquinone — and possibly semiquinones — might be important (32, 42). Catechol, hydroquinone, and phenol have all been shown to induce sister chromatid exchange in cultured human lymphocytes with and without metabolic activation (32). Phenol has usually given negative results in all assays for point mutations. In one study, it was reported to have mutagenic effect at the HGPRT locus in Chinese hamster V79 cells in the presence of microsomal enzymes from mouse liver (38), but it did not produce micronuclei in mice in vivo (44).

Alkane derivatives of benzene, such as toluene (methylbenzene) and xylene (dimethylbenzene), have given only negative results in microbial mutagenicity tests, in accordance with the data on benzene. In general, the mutagenicity of toluene and xylene has not been studied extensively. Chromosome aberrations in the bone marrow of rats have been reported after treat-

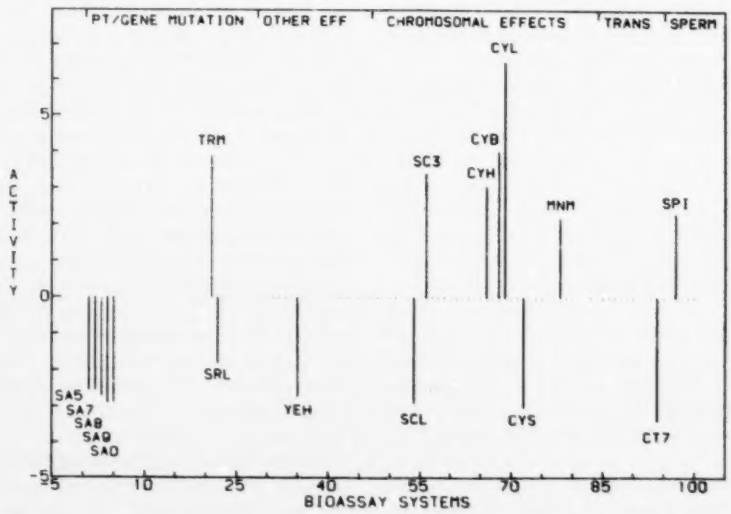

Figure 1. Genetic activity spectrum of benzene. (For an explanation of the codes, see table 1.)

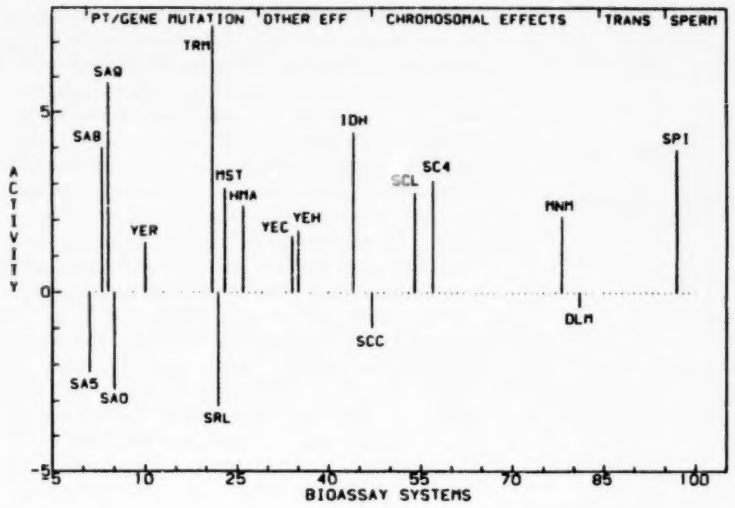

Figure 2. Genetic activity spectrum of trichloroethylene. (For an explanation of the codes, see table 1.)

ment in vivo with toluene of unknown purity (6); no increase in chromosome damage was observed, however, when purified toluene was tested in mice (13). In two studies out of four (2, 11, 12, 25) an increase in the number of chromosome aberrations was found in the peripheral lymphocytes of toluene-exposed rotograph workers. Bauchinger et al (2) also found that rotograph workers have an elevated frequency of sister chromatid exchanges, while Mäki-Paakkanen et al (25) reported no such effect. The evaluation of these human studies is difficult, however, because of possible mixed exposures and because the effect of smoking is not always taken into account. Technical grade xylene increased the number of recessive lethal mutations in *Drosophila melanogaster,* but pure *ortho*-xylene, *meta*-xylene, and ethylbenzene (an impurity in technical xylene) were not mutagenic (8).

Ethylbenzene produced a marginal increase in the incidence of sister chromatid exchanges in human lymphocyte cultures in the absence of metabolic activation (35). Toluene, xylene, and benzene did not induce sister chromatid exchange in this system in the absence of metabolic activation (15), but benzene has been reported to elevate the incidence of sister chromatid exchanges in cultured human lymphocytes both in the presence (31) and absence (7) of S-9 mix.

Chlorobenzene, and the substituted dichlorobenzenes (*ortho-, meta-,* and *para*-), yielded negative results for mutagenicity in *Salmonella typhimurium* (40). *ortho*-Dichlorobenzene has been reported to increase the number of chromosome aberrations in lymphocytes of hospital employees exposed accidentally to this compound (46).

Nitrobenzene has been reported to be nonmutagenic to *S typhimurium* (40) and to be unable to induce unscheduled synthesis of deoxyribonucleic acid (DNA) in an in vivo/in vitro rat hepatocyte assay (30).

Cyclohexane (hexahydrobenzene) also failed to cause mutations in *S typhimurium* tester strains (28).

Benzene can thus induce chromosome damage in several systems after metabolic activation, although it does not appear to be a potent inducer of gene mutations. On the basis of the limited data available, alkyl derivatives of benzene do not seem to be effective clastogens. A possible explanation for this difference between benzene and its alkyl derivatives is the apparent lack of common reactive metabolites.

Vinyl compounds

The basic chemical in the class of vinyl compounds, many of which are used as solvents, is ethylene. No data are available on the mutagenicity of ethylene, but animal experiments indicate that it is converted into ethylene oxide, which is a well-known mutagen and carcinogen (10, 20). The formation of epoxides through microsomal monooxygenation is an important biotransformation route for vinyl compounds. For example, the probable primary reactive metabolite of vinyl chloride, chloroethylene oxide, is formed through microsomal epoxidation (17). Thus, vinyl compounds would be expected to be indirect mutagens. The presence of different substituents at the vinylic carbons, of vinyl compounds, may result in great variation in their mutagenicity, due to differences in the direct reactivity of the substituted compounds, in their metabolic activation or inactivation, or in the reactivity of the ultimate reactive species.

For example, in rats the formation of reactive metabolites from different halogenated ethylenes ranges over two orders of magnitude, so that, for instance, the metabolic rate of vinylidene chloride is 100 times higher than that of vinylidene fluoride. Accordingly

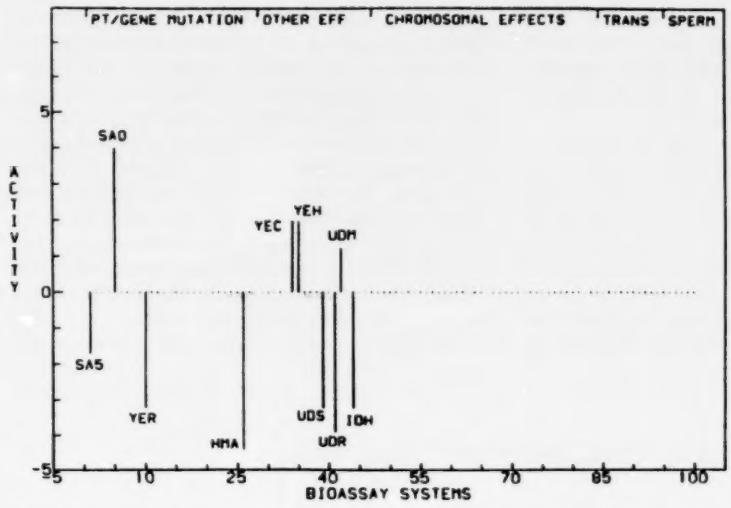

Figure 3. Genetic activity spectrum of tetrachloroethylene. (For an explanation of the codes, see table 1.)

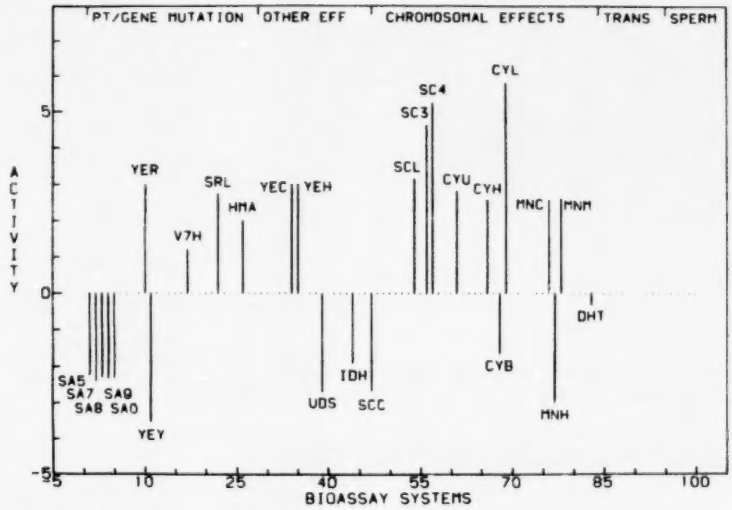

Figure 4. Genetic activity spectrum of vinylbenzene (styrene). (For an explanation of the codes, see table 1.)

vinylidene fluoride is only slightly mutagenic to *S typhimurium* in the presence of metabolic activation, while vinylidene chloride is clearly mutagenic in the same assay (24).

Bonse & Henschler (4) have proposed that the reactivity and toxicity of epoxides derived from individual chloroethylenes depend on the type of chlorine substitution so that epoxides of the symmetrical 1,2-chloroethylenes and tetrachloroethylene would be poor mutagens, as opposed to the epoxides of the asymmetrical vinyl chloride and vinylidene chloride, which are clearly mutagenic. Trichloroethylene is regarded as an exception to this rule, because its asymmetrical epoxide rapidly rearranges to trichloroacetaldehyde (24). The genetic activity spectra of trichloroethylene and tetrachloroethylene show strikingly divergent results. (See figures 2 and 3.) The only concordant positive results obtained for the two compounds were for enhanced gene conversion and mitotic recombination in yeast. Concordant negative results were obtained only in *S typhimurium* TA 1535. Conflicting results were obtained for reverse mutation in *S typhimurium* TA100 and in *Saccharomyces cerevisiae* in vitro and in a host-mediated assay. The proportion of positive responses was higher with trichloroethylene than with tetrachloroethylene.

Some vinyl compounds can also be oxidized by non-microsomal routes. For instance, styrene is converted in human blood to styrene 7,8-oxide by the action of erythrocytic oxyhemoglobin (3, 43). As a consequence, styrene induces chromosome damage in cultured mammalian cells if erythrocytes are included in the assay

(33, 36). However, styrene gives negative or only slightly positive results in mutagenicity tests employing S-9 mix for metabolic activation (45). Taken together, there is sufficient evidence that styrene is active in short-term tests (19). (See figure 4.)

Studies on the induction of sister chromatid exchange in cultured human lymphocytes suggest that the erythrocyte-mediated oxidation of vinyl compounds depends on the substitution of the vinyl group (33, 35, 36). Erythrocytes seem to be important for the induction of sister chromatid exchange by styrene, ring-methylated styrenes (isomers of vinyl toluene) and for *trans*-beta-methylstyrene, but not for vinyl acetate, acrylonitrile, acrylamide, and N-vinyl-2-pyrrolidone (33).

Epoxides are not the only potential reactive intermediates of vinyl compounds. Vinyl chloride, for example, is also converted to chloroacetaldehyde, which is mutagenic but is not considered as important as the epoxide chloroethylene oxide (17, 24). In certain cases aldehydes seem to be the critical intermediates. Vinyl acetate is broken down very rapidly in biological systems to acetaldehyde (9, Pfäffli & Norppa, unpublished data), which is mutagenic in several test systems. (See figure 5.) The production of acetaldehyde probably explains the efficient induction of sister chromatid exchange and chromosome aberrations by vinyl acetate in cultured human lymphocytes and Chinese hamster ovary cells (26, 33, 34). The formation of chromosome lesions after treatment with vinyl acetate does not require external microsomal preparations, although the use of rat liver S-9 mix does enhance the induction of sister chromatid exchange by

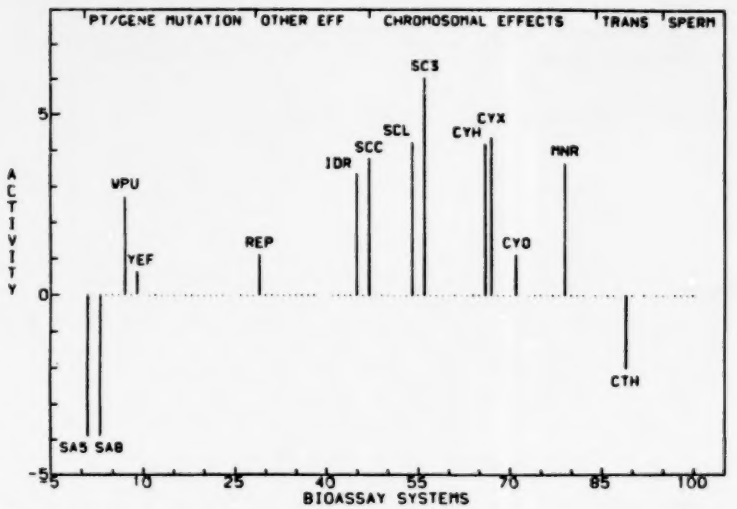

Figure 5. Genetic activity spectrum of acetaldehyde. (For an explanation of the codes, see table 1.)

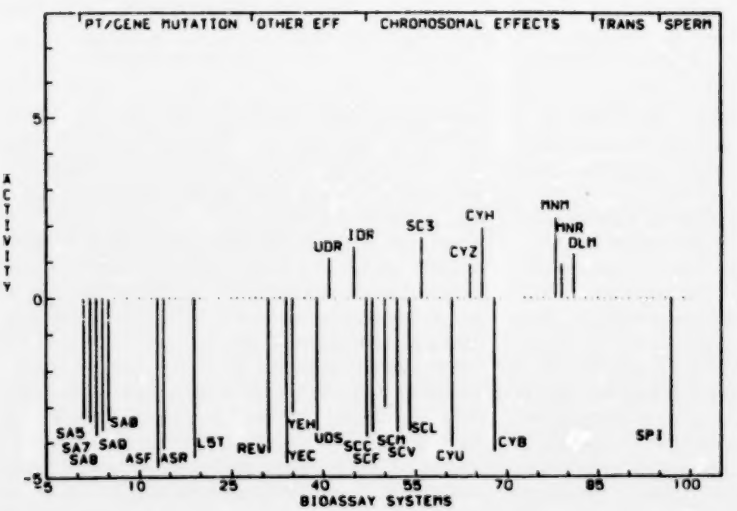

Figure 6. Genetic activity spectrum of ethanol. (For an explanation of the codes, see table 1.)

vinyl acetate in Chinese hamster ovary cells (Norppa, unpublished data). Vinyl acetate may not be the only vinyl compound that is transformed rapidly into an aldehyde; in theory, other vinyl esters could also be expected to go through this process.

Ethanol

Ethanol is used extensively as a solvent and is one of the 50 chemicals produced in largest volume in the United States (47).

Ethanol has been reported to interfere with mitotic segregation and to cause chromosome missegregation and nondisjunction in *Aspergillus nidulans* (21). It is not mutagenic in bacterial assays for point mutations (28) or in yeast (22), and it did not produce sister chromatid exchanges in vitro in most of the mammalian cells studied (37), although a positive response was reported in mouse embryo after in utero exposure (5). Chromosome aberrations have been reported in one study with human blood lymphocytes in vitro (1) and in another with human fibroblasts (29). In addition, ethanol has been observed to induce micronuclei in mice and rats (1).

Alcoholics have a higher frequency of chromosome aberrations in their peripheral blood lymphocytes than nonalcoholics (37), but whether this difference is causally associated with ethanol is not yet clear.

It has been suggested that ethanol may act on the spindle apparatus in mice and induce nondisjunction at conception (23). The genetic activity spectrum of ethanol is given in figure 6.

Conclusions

Of the solvents that are in general use and that are either related to benzene or have an olefinic double bond, such as trichloroethylene, only a few have been demonstrated beyond any doubt to be mutagenic. Benzene is clearly clastogenic, causing chromosome aberrations in mammalian cells both in vivo and in vitro. Close analogues such as toluene, xylenes, and various chlorinated benzenes have been studied to a very limited extent, and the existing data should be considered inadequate to make a final judgement on the activity of these chemicals in short-term tests.

With regard to the vinyl compounds, there is sufficient evidence that acrylonitrile, styrene, and vinyl chloride are active in short-term tests (20). However, the genetic activity of vinyl chloride and acrylonitrile is not similar. Acrylonitrile resembles epichlorohydrin in terms of activity in short-term tests, and vinyl chloride resembles ethylene oxide (15). Technical grade trichloroethylene is also active in a wide spectrum of genetic tests. The proportion of positive results with trichloroethylene is clearly greater than with tetrachloroethylene.

Although there is sufficient evidence that acetaldehyde, the primary metabolite of ethanol, is active in short-term tests (21), the evidence that ethanol itself is mutagenic is weak. (See figure 6.) Assays for point mutations have given negative results. Cytogenetic tests for nondisjunction in yeast and some preliminary studies with mouse embryos suggest that ethanol may act on the spindle apparatus.

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References

1. Badr FM, Asker RL, Hussain FH. Evaluation of the mutagenic effects of ethyl alcohol by different techniques. In: Gross MM, ed. Alcohol intoxication. Volume IIIA (Biological effects of ethanol). Plenum, New York, NY 1977, pp 25—46.
2. Bauchinger M, Schmid E, Dresp J, Kolin-Gerresheim J, Hauf R, Suhr E. Chromosome changes in lymphocytes after occupational exposure to toluene. Mutat Res 102 (1982) 439—445.
3. Belvedere G, Tursi F. Styrene oxidation to styrene oxide in human blood erythrocytes and lymphocytes. Res Commun Chem Pathol Pharmacol 33 (1981) 273—282.
4. Bonse G, Henschler D. Chemical reactivity biotransformation and toxicity of polychlorinated aliphatic compounds. CRC Crit Rev Toxicol 5 (1976) 395—409.
5. Czajka MR, Tucci SM, Kaye GI. Sister chromatid exchange frequency in mouse embryo chromosomes after *in utero* ethanol exposure. Toxicol Lett 6 (1980) 257—261.
6. Dean BJ. Genetic toxicology of benzene, toluene, xylenes and phenols. Mutat Res 47 (1978) 75—97.
7. Diaz N, Fijtman V, Carricarte L, Braier L, Diez J. Effects of benzene on sister chromatid exchange in human lymphocytes. Environ Mutagenesis 1 (1979) 177.
8. Donner M, Mäki-Paakkanen J, Norppa H, Sorsa M, Vainio H. Genetic toxicology of xylenes. Mutat Res 74 (1980) 171—172.
9. Filov VA. On the fate of complex esters of vinyl alcohol and fatty acids in the organism. Gig Tr Prof Zabol 3 (1959) 42—46.
10. Filser JG, Bolt HM. Exhalation of ethylene oxide by rats on exposure to ethylene. Mutat Res 120 (1983) 57—60.
11. Forni A, Pacifico E, Limonata A. Chromosome studies in workers exposed to benzene or toluene or both. Arch Environ Health 22 (1971) 373—378.
12. Funes-Cravioto F, Kolmodin-Hedman B, Lindsten J, Nordenskjöld M, Zapata-Gayon C, Lambert B, Nordberg E, Olin R, Swensson Å. Chromosome aberrations and sister chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. Lancet 2 (1977) 322—325.
13. Gad-El-Karim MM, Harper BL, Legator MS. Modifications in the myeloclastogenic effect of benzene in mice with toluene, phenobarbital, 3-methylcholanthrene, Arochlor 1254 and SKF-525A. Mutat Res 135 (1984) 225—243.
14. Garrett NE, Stack HF, Gross MR, Waters MD. An analysis of the spectra of genetic activity produced by

known or suspected human carcinogens. Mutat Res 134 (1984) 89—111.
15. Gerner-Smidt P, Friedrich U. The mutagenic effect of benzene, toluene, and xylene studied by the SCE technique. Mutat Res 85 (1978) 313—316.
16. Goldstein BD. Benzene is still with us. Am J ind Med 4 (1983) 585—587.
17. Green T, Hathaway DE. The chemistry and biogenesis of the S-containing metabolites of vinyl chloride in rats. Chem Biol Interactions 17 (1977) 137—150.
18. International Agency for Research on Cancer. Some industrial chemicals and dyestuffs. Lyon 1982, pp 93—148. (IARC monograph on the evaluation of the carcinogenic risk of chemicals to humans, volume 29).
19. International Agency for Research on Cancer. Chemicals, industrial process and industries associated with cancer in humans (IARC monographs, volumes 1 to 29). Lyon 1982, pp 25—26, 229—233, 260—262. (IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, supplement no 4).
20. International Agency for Research on Cancer. Some allyl and allylic compounds, aldehydes, epoxides and peroxides. Lyon (in press). (IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, volume 36).
21. Käfer E. Disruptive effects of ethyl alcohol on mitotic chromosome segregation in diploid and haploid strains of *Aspergillus nidulans*. Mutat Res 135 (1984) 53—75.
22. Kappas A, Bridges BA. Induction of point mutations of benomyl in DNA-repair-deficient *Aspergillus nidulans*. Mutat Res 9 (1981) 115—118.
23. Kaufman MH. Ethanol-induced chromosomal abnormalities at conception. Nature 302 (1983) 258—260.
24. Laib KJ. Specific covalent binding and toxicity of alipnatic halogenated xenobiotics. In: Beckett AH, Gorrod JW, ed. Reviews on drug metabolism and drug interactions. Volume IV, no 1. Freund Publishing House Ltd, London 1982, pp 1—48.
25. Mäki-Paakkanen J, Husgafvel-Pursiainen K, Kalliomäki P-L, Tuominen J, Sorsa M. Toluene-exposed workers and chromosome aberrations. J Toxicol Environ Health 6 (1980) 775—781.
26. Mäki-Paakkanen J, Tursi F, Norppa H, Järventaus H, Sorsa M. Vinylacetate is a potent inducer of chromosome damage in cultured mammalian cells. Mutat Res 130 (1984) 249.
27. Maltoni C, Conti B, Cotti G. Benzene: A multipotential carcinogen: Results of long-term bioassays performed at the Bologna Institute of Oncology. Am J ind Med 4 (1983) 589—630.
28. McCann J, Choi E, Yamasaki E, Ames, BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci USA 72 (1975) 5135—5139.
29. Meisner LF, Inhorn SL, Nielsen PM. Chemically induced chromatid breaks: Toxigenic or mutagenic. Mamm Chromosomes Newsl 11 (1970) 69—70.
30. Mirsalis JC, Hamm TE Jr, Sherrill JM, Butterworth BE. The role of gut flora in the genotoxicity of dinitrotoluene. Nature 295 (1982) 322—323.
31. Morimoto K. Induction of sister chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res 43 (1983) 1330—1334.
32. Morimoto K, Wolff S, Koizumi A. Induction of sister-chromatid exchanges in human lymphocytes by microsomal activation of benzene metabolites. Mutat Res 119 (1983) 355—360.
33. Norppa H, Tursi F. Erythrocyte-mediated metabolic activation detected by SCE. In: Tice RR, Hollaender A, ed. Sister-chromatid exchanges: 25 years of experimental research, Part B, genetic toxicology and human studies. Plenum, New York, NY 1984, pp 547—560.
34. Norppa H, Tursi F, Einistö P. Erythrocytes as a metabolic activation system in mutagenicity tests. In: Institut National de la Santé et de la Recherche Médicale. Proceedings of the 13th Annual Meeting of European Environmental Mutagen Society, Montpellier, 5—9 September 1983. (in press). (INSERM symposia series).
35. Norppa H, Vainio H. Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. Mutat Res 116 (1983) 379—387.
36. Norppa H, Vainio H, Sorsa M. Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. Cancer Res 43 (1983) 3579—3582.
37. Obe G, Ristow HJ. Mutagenic, cancerogenic and teratogenic effects of alcohol. Mutat Res 65 (1979) 229—259.
38. Paschin UV, Bahitova LM. Mutagenicity of benzo(a)pyrene and the antioxidant phenol at the HGRPT locus of V79 Chinese hamster cells. Mutat Res 104 (1982) 389—393.
39. Sawahata T, Neal RA. Biotransformation of phenol to hydroquinone and catechol by rat liver microsomes. Mol Pharmacol 23 (1983) 453—460.
40. Shimizu M, Yasui Y, Matsumoto N. Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium* — A series of chloro- or fluoro-nitrobenzene derivatives. Mutat Res 116 (1983) 217—238.
41. Styles JA, Richardson CP. Cytogenetic effects of benzene: Dosimetric studies on rats exposed to benzene vapour. Mutat Res 135 (1984) 203—205.
42. Tunek A, Platt KL, Przybylshi M, Oesch F. Multi-step metabolic activation of benzene: Effect of superoxide dismutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. Chem Biol Interactions 33 (1980) 1—17.
43. Tursi F, Samaia M, Salmona M, Belvedere G. Styrene oxidation to styrene oxide in human erythrocytes is catalyzed by oxyhemoglobin. Experientia 39 (1983) 593—594.
44. Wild D, King M-T, Eckhardt K. Cytogenic effect of ortho-phenylenediamine in the mouse Chinese hamster and guinea pig and of derivatives evaluated by the micronucleus test. Toxicology 43 (1980) 249—255.
45. World Health Organization. Styrene. Geneva 1983, pp 1—123. (IPCS environmental health criteria 26).
46. Zapata-Gayon C, Zapata-Gayon N, Gonzales-Angulo A. Clastogenic chromosomal aberrations in 26 individuals accidentally exposed to ortho-dichlorobenzene vapors in the National Medical Center in Mexico City. Arch Environ Health 37 (1982) 231—235.
47. _____ . Production by the US chemical industry: Output down for chemicals, related products. Top 50 chemicals. Chem Eng News June 13 (1983) 29.

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